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Virulence reaction norms across a food gradient

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Host–parasite interactions involve competition for nutritional resources between hosts and the parasites growing within them. Consuming part of a host's resources is one cause of a parasite's virulence, i.e. part of the fitness cost imposed on the host by the parasite. The influence of a host's nutritional conditions on the virulence of a parasite was experimentally tested using the mosquito *Aedes aegypti* and the microsporidian parasite *Vavraia culicis*. A condition-dependent expression of virulence was found and a positive relation between virulence and transmissibility was established. Spore production was positively influenced by host food availability, indicating that the parasite's within-host growth is limited by host condition. We also investigated how the fitness of each partner varied across the nutritional gradient and demonstrated that the sign of the correlation between host fitness and parasite fitness depended on the amount of nutritional resources available to the host.

Keywords: *Aedes aegypti*; *Vavraia culicis*; microsporidia; transmission; condition-dependent virulence

1. INTRODUCTION

Competition for resources between host and parasite is one of the causes of a parasite's virulence, defined as the negative effects on host fitness, as parasites consume part of the resources normally devoted to a host's growth, maintenance and/or reproduction. Parasites can affect hosts simply by reducing the resources available for growth or maintenance. For example, Siva-Jothy & Plaistow (1999) found reduced lipid accumulation in damselflies parasitized by eugregarines. Parasites can also manipulate a host's pattern of resource allocation to increase the resources allocated to the area from which the parasite directly benefits. This is one of the possible explanations for parasitic castration, which may be adaptive for the parasite because it decreases the proportion of resources devoted to host reproduction while increasing the amount of resources to which the parasites have access (Minchella 1985; Thompson & Kavaliers 1994). Another way to increase the total amount of resources a parasite can access is to delay the host's sexual maturation and thus increase its growth period (Thompson & Kavaliers 1994).

The shape of the relation between host resources (or richness of the environment) and the fraction of these that the parasite uses affects the relationship linking richness of the environment and virulence (Jokela *et al.* 1999) and that linking virulence and parasite replication. Food-condition-dependent expression of virulence has already been reported in some host–parasite systems, for example in bumble-bees infected by trypanosomes (Brown *et al.* 2000) and snails infected by trematodes (Jokela *et al.* 1999). In both studies, two food treatments were compared: *ad libitum* food and starvation. Mortality caused by the parasite was higher in starvation treatments than *ad libitum* treatments for the same infective dose. Moreover, Ferguson & Read (2002) reported a greater reduction in longevity for the mosquito *Anopheles stephensi* infected with certain *Plasmodium chabaudi* genotypes when reared in glucose-deprived conditions than when they were reared with continuously available glucose water.

We used the relationship of the mosquito *Aedes aegypti* and the microsporidian parasite *Vavraia culicis* to address the following questions. (i) Does the parasite's virulence vary with the richness of the environment? (ii) In view of the differences in the life-history traits of male and female hosts, does the parasite affect each sex equally? (iii) Are the virulence and transmissibility of the parasite correlated? (iv) Is within-host parasite growth altered by host resource availability? (v) How do the fitnesses of the host and parasite vary with resource availability?

2. MATERIAL AND METHODS

(a) *Experimental system*

Vavraia culicis naturally infects several genera of mosquito (Weiser 1980). Spores of *V. culicis* are ingested by host larvae along with their food, and they infect gut cells. Within host cells, the parasite proliferates as it passes through a series of developmental stages before producing its spores. Spore-laden cells rupture liberating spores to infect other gut cells or fat body cells. No known component of an insect's immune system can neutralize this intracellular parasite. The parasite's transmission is horizontal and its spores do not resist desiccation (Kelly *et al.* 1981). Transmission thus occurs mainly from larva to larva, either by spores released in faeces or following larval death (Becnel & Andreadis 1999, p. 455).

Aedes aegypti is a subtropical mosquito whose larvae grow in natural or artificial containers (Southwood *et al.* 1972). These sites show temporal variation in size and food availability. Larvae feed by filtering water, while pupae do not feed. Below a certain resource threshold, larvae do not pupate but remain as larvae; the extended larval period depends on their accumulated reserves (Gilpin & McClelland 1979). The pupation threshold depends on various factors such as temperature and strain, but within a strain and at the same temperature, the threshold is

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higher for females than males. Females emerge as larger and heavier adults than males and thus require more food to build their body. This size difference stems from different selection pressures acting on males and females, owing to their different roles in reproduction (Rice & Chippindale 2001; Bedhomme *et al.* 2003). The different food requirements of males and females and the consumption of resources by the parasite lead to the expectation that *V. culicis* should affect female growth more than male growth and bias the adult sex ratio towards males.

(b) *Experimental design*

The strain of *Ae. aegypti* used is derived from a large number of eggs collected in Brazil (Tingua) and provided by Ricardo Lourenço de Oliveira of the Instituto Oswaldo Cruz (Rio de Janeiro). It had been reared in standardized and outbred conditions (3000 reproductive adults in each generation) in our laboratory for three generations at the time of the experiment. The spores of *V. culicis* used were derived from a stock isolated from *Ae. albopictus* in Florida and were provided by Dr J. J. Becnel (United States Department of Agriculture, Gainesville, FL, USA).

Recently hatched larvae were split into groups of 60 and each group was put in a Petri dish (diameter of 55 mm) containing 10 ml of softened water. To 11 dishes, we added 2×10^4 *V. culicis* spores per larva in 0.1 ml of softened water. To the 10 control Petri dishes, we added 0.1 ml of water. To each dish, we added 3.6 mg of Tetramin (fish food). Spores and larvae were kept in contact for 24 h. After this infection period, larvae were rinsed and each larva was transferred to an individual *Drosophila* vial (diameter of 25 mm × 95 mm) containing 5 ml of softened water. Contact between larvae and spores was restricted to 24 h to synchronize the age structures of the infections. Control and exposed larvae were equally distributed along a gradient of food resources involving nine food treatments: 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.6 and 3.0 mg of Tetramin per larva. Food was distributed after each 24-h-old larva was put in its individual vial. At the lowest level in the food gradient almost no larvae achieve complete development and at the highest level there is almost 100% emergence. This gradient was established in a preliminary experiment with this strain. There were 60 larvae in each exposure × food treatment, yielding 1080 larvae. Vials were arranged in racks of 4 × 10 vials. Each column of a rack corresponded to a food level. Two rows were filled with exposed individuals and the remaining two with controls. The experiment took place in a room maintained at 25 °C and a photoperiod of 12 L : 12 D.

Vials were checked every 24 h and the ages at pupation and death were noted. When pupation occurred, pupae were transferred to 5 ml of clean water and the vials covered with a fine nylon gauze. The time of adult emergence was noted to within 24 h and the mosquito's sex was recorded. Adults had access only to water and subsequently starved to death. Age at death was recorded and dead individuals were transferred to 1.7 ml plastic vials. Dead adults were kept at 65 °C for a minimum of 12 h and their dry weight was recorded to an accuracy of 1 μg (with a Mettler Toledo MX 5 microbalance). One wing was removed from each adult and the distance between the alula notch and wing tip was measured to a precision of 0.03 mm using a dissecting microscope fitted with a graduated eyepiece. For all exposed individuals and 81 control individuals having an age at death of more than 8 days, spore load was evaluated by homogenizing the body of the individual in 0.2 ml of water and counting the number of spores with a Neubauer cell counter and phase-contrast light microscope. Individuals exposed to infection and not found to be harbouring spores at least 8 days later were assumed to be uninfected and were excluded from the analyses (130/521, 25.0%). Between each step of data collection, dead mosquitoes were conserved at −20 °C.

(c) *Statistical analyses*

Statistical analyses were performed with JMP, v. 3.2.2 (SAS Institute 1997). To evaluate the influences of food level and parasitism on the probability of emergence, we used a dose–response curve analysis (Motulsky 1999). At each food level, we calculated the proportion of individuals emerging and fitted curves using the general equation for a dose–response curve: $\text{response} = b + (t - b)/(1 + 10^{((\text{EC50} - \log(\text{food level})) \times h)})$. In this equation, b represents the lowest level of the response, t the highest level of the response, EC50 the food level for which the response is exactly midway between b and t, and h the slope of the curve at EC50. Here, b was set to zero because in environments without food the probability of emergence will be zero; t is the proportion of individuals emerging at *ad libitum* food levels (*ca.* 1.0). EC50 is the food quantity that allows half of the individuals to emerge.

We first constructed a combined model with all the data and estimated values of the three parameters t, EC50 and h. We then made a separate model allowing different values of t, EC50 and h to be fitted for infected and control treatments, thus evaluating six parameters. An F-test compared the relative difference in the sums of the squares of the two models $((SS_{\text{combined model}} - SS_{\text{separate model}})/SS_{\text{separate model}})$ with the relative difference in the degrees of freedom in the two models $((\text{d.f.}_{\text{combined model}} - \text{d.f.}_{\text{separate model}})/\text{d.f.}_{\text{separate model}})$ (Motulsky 1999). Thus, we estimated the overall probability of emergence as a function of food availability and then tested whether including 'infection status' explained a significantly greater amount of the observed variation. Evaluation of these parameters and their confidence intervals allowed us to test which curve parameters were modified by the infection treatment.

The same approach was applied to analyse sex-ratio data. We fixed t as 1 (as only males emerge in very-low-food treatments) and b as 0.5 (as the sex ratio was equal at *ad libitum* food levels). We thus evaluated sex ratio as a function of two (in the combined model) or four (in the separate model) parameters.

We also wanted to know the effects of parasitism on quantitative life-history traits of the host and whether these changed with the food resources available to the host or with the differential food requirements of each sex. To do so, we performed three-way analyses of variance (ANOVA) on the life-history traits of larval developmental time, adult longevity (day of death − day of emergence), proportion of time spent in the aquatic environment (day of emergence/day of death), wing length and starved adult dry weight. Food, parasitism and sex were treated as fixed factors and an effect block (a group of four racks) was taken as a random factor to take into account microenvironmental variation. These analyses included only individuals from food levels in which a sufficient number of mosquitoes reached adulthood (1.6 mg or more). Developmental-time data were log transformed and wing-length data were Box–Cox transformed to meet ANOVA requirements. Data on starved adult dry weight were analysed by a non-parametric ANOVA test (Sokal & Rohlf 2001), owing to departures from the conditions required for a parametric ANOVA.

We wanted to test whether the parasite's reproduction (spore production) was influenced by the amount of resources available to its host (food treatment). Spore production, however, is also

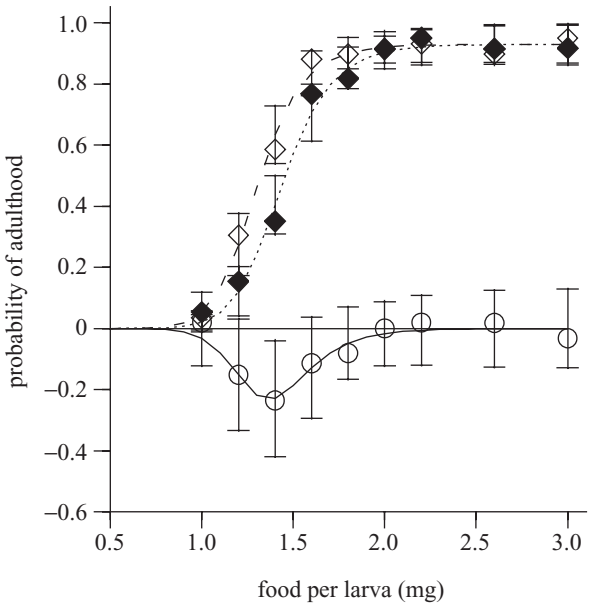

Figure 1. Probability of emergence of control and infected hosts across food treatments. Open diamonds represent control treatments and filled diamonds represent infected treatments. The dashed line is the predicted curve for controls and the dotted line is the predicted curve for infected treatments. Circles represent the difference between predicted values for control and infected treatments. The error bars represent confidence intervals around the predicted values.

affected by the time an individual host has lived (total longevity from hatching to death). These two variables are not independent, since individuals receiving more food as larvae also live longer (Agnew *et al.* 2002). To avoid this problem, we verified that infection did not affect the total length of life (from hatching to death). We took the residuals of the regression of total longevity on food level. The residuals from this analysis were used as a covariate in a regression of log(number of spores) as a function of food. These analyses were applied to individuals dying as larvae in the 1.0, 1.2 and 1.4 mg food treatments and separately to individuals dying as adults in the higher-food treatments, as the mean days of death for larvae (23.5 days) and adults (13.7 days) were different.

3. RESULTS

In high-food treatments (1.6 mg or more), the majority of individuals reached adulthood in 8–10 days. In lower-food treatments (1.4 mg or less), a fraction of individuals reached adulthood by 10 days, with the rest of the population remaining as larvae for longer (10–20 days). The dose–response analysis indicated that the probability of emergence increased with food availability (combined model: $F_{3,15} = 19.91$, $p < 0.001$) and was also influenced by infection (improved fit with separate curves: $F_{3,12} = 6.26$, $p < 0.01$; figure 1). The curves fitted to infected and control treatments differed significantly only in the EC50 parameter, which was 0.1145 (s.e. 0.0090) for the control curve and 0.1646 (s.e. 0.0081) for the infected curve. This indicates that the quantities of food required for half of the individuals to emerge were 1.30 and 1.46 mg, respectively. Emergence probabilities estimated by the models at each food level showed a significantly lower probability of emergence for the 1.4 mg infected treatment than for the control treatment. At 1.2 mg, the difference in emergence probability was marginally significant (figure 1).

A strongly male-biased sex ratio in low-food treatments decreased to equality as food availability increased. Contrary to our expectation, infection had no significant effect on sex ratios. The comparison of the combined and separate models gave an F-ratio of $F_{2,12} = 1.18$ ($p > 0.5$).

Food, sex and infection had significant effects on adult life-history traits. Males developed faster ($F_{1,561} = 105.08$, $p < 0.001$), lived longer as adults ($F_{1,561} = 1302.99$, $p < 0.001$) and were smaller ($F_{1,561} = 2688.26$, $p < 0.001$) and lighter ($H_1 = 175.77$, $p < 0.001$) than females. As food became more abundant, mosquitoes developed faster ($F_{5,561} = 2.96$, $p = 0.012$), lived longer as adults ($F_{5,561} = 67.44$, $p < 0.001$) and were larger ($F_{5,561} = 204.50$, $p < 0.001$) and heavier ($H_5 = 40.95$, $p < 0.001$). Infected individuals developed more slowly ($F_{1,561} = 42.23$, $p < 0.01$; figure 2*a*), lived for less time as adults ($F_{1,561} = 13.03$, $p < 0.001$) and were smaller ($F_{1,561} = 50.05$, $p < 0.001$) and lighter ($H_1 = 6.75$, $p < 0.01$; figure 2*b*) than control adults. Interactions between the factors sex and food were significant for adult longevity ($F_{5,561} = 20.96$, $p < 0.001$; figure 2*c*) and wing length ($F_{5,561} = 8.90$, $p < 0.001$), with a greater increase in longevity for males and body size for females as the food availability increased. The interaction between sex and infection was significant for adult longevity ($F_{1,561} = 10.68$, $p = 0.001$; figure 2*c*): longevity was similar for infected and control females, whereas longevity was reduced for infected males compared with control males. The interaction between food and infection was not significant for any trait analysed.

Infection increased the proportion of total life spent in the aquatic environment ($F_{1,575} = 25.82$, $p < 0.001$). There was a significant interaction between infection and sex for this trait ($F_{1,575} = 6.65$, $p = 0.010$): the increase in the proportion of aquatic life was greater for males (from 62.5% to 65.5%) than females (from 76.7% to 77.6%).

Host food conditions had a positive influence on spore production. A preliminary analysis indicated that infection had no significant effect on the total longevity of either larvae ($H = 1.31$, $p > 0.1$) or adults ($H = 3.16$, $p > 0.05$). For larvae, the partial regression was log(number of spores) = 3.11 + (0.91 × food) + (0.024 × residual longevity), with significant effects for food ($F_{1,116} = 10.42$, $p = 0.002$) and residual longevity ($F_{1,116} = 4.56$, $p = 0.035$). For adults, the partial regression was log(number of spores) = 3.46+ (0.20 × food) + (0.15 × residual longevity), with significant effects for food ($F_{1,221} = 15.16$, $p < 0.001$) and residual longevity ($F_{1,221} = 57.27$, $p < 0.001$). When food abundance increased, more spores were produced and this influence was strongest for the individuals dying as larvae in low-food treatments (figure 3).

4. DISCUSSION

(a) *Costs of parasitism for the host*

Vavraia culicis had both condition-dependent and condition-independent effects on its host. The condition-dependent effect was expressed for host emergence

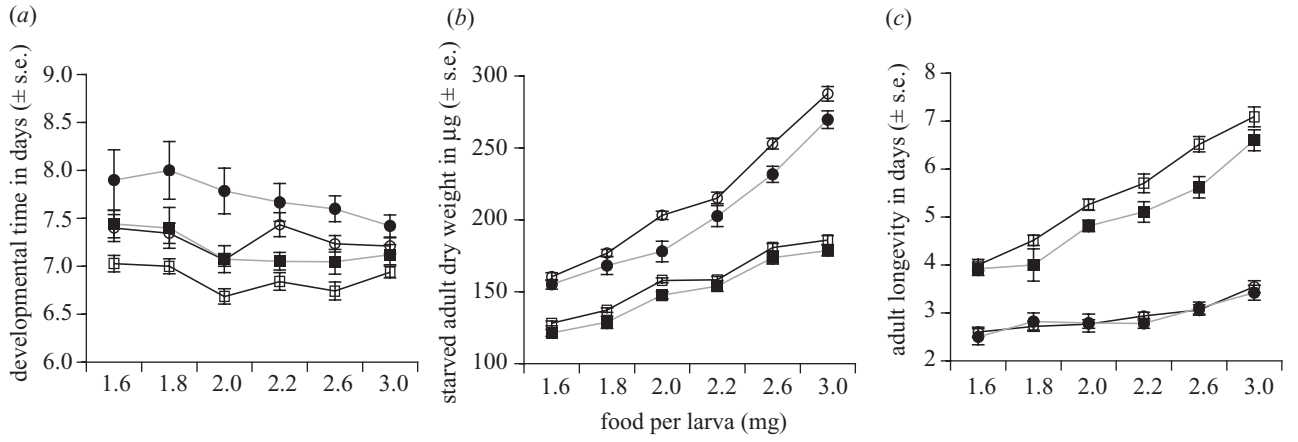

Figure 2. (*a*) Developmental time in days (± s.e.); (*b*) starved adult dry weight in µg (± s.e.); and (*c*) adult longevity in days (± s.e.). Squares represent males and circles represent females; open symbols represent control treatments and filled symbols represent infected treatments.

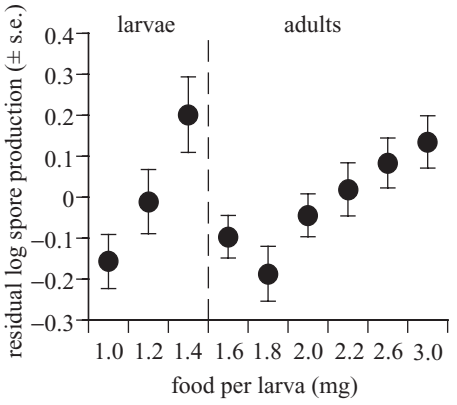

Figure 3. Influence of larval food availability on spore production for individuals dying as larvae (food treatments 1.0–1.4 mg) and as adults (food treatments 1.6–3.0 mg). Residual spore production reflects spore production after taking into account the age at which individuals died.

probability. *Vavraia culicis* increased the quantity of food necessary for half of its host population to emerge. This shift in EC50 for infected individuals, combined with the absence of differences in other curve parameters, resulted in a difference in emergence probabilities at intermediate food levels (figure 1). At very low food levels, the probability of emergence was close to zero and the parasite's virulence had no opportunity to be expressed. At high food levels, the probability of emergence approached 100% in both infected and uninfected treatments. The host's probability of emergence was reduced by the parasite only at intermediate food levels. The food gradient we used had a more qualitative than quantitative value: our goal was to test whether virulence depended on food conditions and not to quantify the exact food requirements for this component of virulence to be expressed. In different host populations or environments, EC50 values are likely to be different but the qualitative trends we observed are likely to remain the same.

The condition-dependent virulence could explain the absence of effects of *V. culicis* on larval mortality reported in studies where mosquito larvae were in optimal conditions (Reynolds 1970; Agnew *et al.* 1999). As larval resource limitation is a major factor regulating populations of container-developing mosquitoes (Washburn 1995), *Ae. aegypti* populations may frequently be in conditions where an infection by *V. culicis* would cause a reduction in the probability of emergence. In general, for dose–response curves, such an increase in the EC50 parameter, shifting the curve to the right, indicates the presence of a competitive antagonist (Motulsky 1999). *Vavraia culicis* apparently acts as an antagonist by consuming host resources and thus increasing the threshold for pupation.

Interactions between parasite and host resources can take many forms: a parasite could simply reduce the total resources that the host effectively has access to; it could also modify the pattern of resource allocation to different functions of the organism—this is typically the case for castrating parasites (Minchella 1985). Parasites can also modify resource allocation to different types of reserves. This has been found for *Gammarus pulex* infected by the acanthocephalan parasite *Pomphorhynchus laevis* (Plaistow *et al.* 2001): infected gravid females had less lipids than control females, and infected males and females had more glycogen than control individuals. In our system, we do not know whether pupation in *Ae. aegypti* is related to a minimal weight or to a minimal content of one type of resource, as suggested by Chambers & Klowden (1990). The development of *V. culicis* may thus decrease the total amount of resources available to its host and at intermediate levels of nutritional availability this places the host under the threshold required for pupation, be this a minimal weight or a minimal content of one biochemical type of reserve. The absence of effects of *V. culicis* on the probability of emergence at high food levels is probably the result of larval hosts reaching the conditions required for pupation and emergence despite some of their resources being consumed by the parasite.

The costs of parasitism were also observed in adult life-history traits. Infected hosts had a longer developmental time and emerged as smaller and lighter adults with a reduced longevity. Each of these trends is associated with lower fitness (Clements 1992). However, there was no significant interaction between food level and infection, indicating that the parasite's costs to adult traits did not depend on the host's nutritional state. An explanation for this lack of effect is that above a certain level of resources

the multiplication of the parasite is limited by something other than resource abundance. This explanation is also supported by the steeper relation between food availability and spore production for low than for high food levels (figure 3).

The analysis of adult life-history traits showed known differences between males and females: males emerged earlier and were smaller and lighter than females. These characteristics and the differential reactions of male and female life-history traits, particularly longevity and wing length, to food abundance stem from the different ways in which these traits are related to each sex's fitness (Bedhomme *et al.* 2003). Females are selected to be larger because of the relationship between size and fecundity (Colless & Chellapah 1960), while males are selected to emerge earlier owing to the advantages of a short developmental time in male–male competition for access to females in synchronized populations (Clements 1992). *Aedes aegypti* populations are often synchronized in the field: eggs laid at the water–sediment interface hatch synchronously when they are subsequently immersed (Clements 1992).

We also found that males lived longer as adults than did females. This result was probably caused by the conditions in which this measurement was made: adults were in individual tubes, did not use a lot of energy in flying and had no sexual activity. In a paper by Hausermann & Nijhout (1975), fig. 1 suggests that sexual activity reduces male longevity in *Ae. aegypti*. This probably explains the apparent contradiction between our results and those often observed in population cages (Lansdown & Hacker 1975) or the field (Trpis & Hausermann 1986), where sexual activity is unfettered and females typically live longer than males.

The different reaction norms of male and female traits to food conditions, the different thresholds of food requirements for pupation between the sexes and the fact that the parasite's costs are, at least partly, the result of consumption of part of its host's resources are good reasons to think that the impact of parasites should be different for males and females. In particular, we predicted that the sex ratio would be biased towards males in infected treatments compared to uninfected treatments. This prediction was not verified by our data, perhaps because the number of individuals in each treatment was too low to estimate sex ratios precisely. The only significant interaction between the factors of sex and infection was for adult longevity: male longevity was reduced by infection whereas female longevity was not. A possible explanation for this interaction is that the parasite preferentially consumed a biochemical resource that enabled males to live longer under our experimental conditions. Such resources are likely to be lipids (Briegel 1990).

(b) *Implications of virulence for the parasite*

From the parasite's point of view, reducing the probability of host emergence is beneficial as it increases the chances of the host dying in the aquatic environment, the medium in which the parasite's spores remain viable and have the highest probability of transmission. Moreover, increases of larval lifespan and decreases of adult lifespan caused by the parasite make the proportion of larval life greater in infected hosts. The parasite may thus increase the proportion of the host's life that is spent in a developmental stage, in which the probability of transmission is high. Spores may be found in water because infected larvae die or because the corpses of infected adults end up in water. Spores within infected adults must have a non-zero contribution to the parasite's fitness, at least during the colonization of new sites. Unless the bodies of these adults return to their larval site, the spores they contain will not contribute to the within-site transmission success of the parasite; this will be maintained by host individuals dying as larvae or pupae within the site.

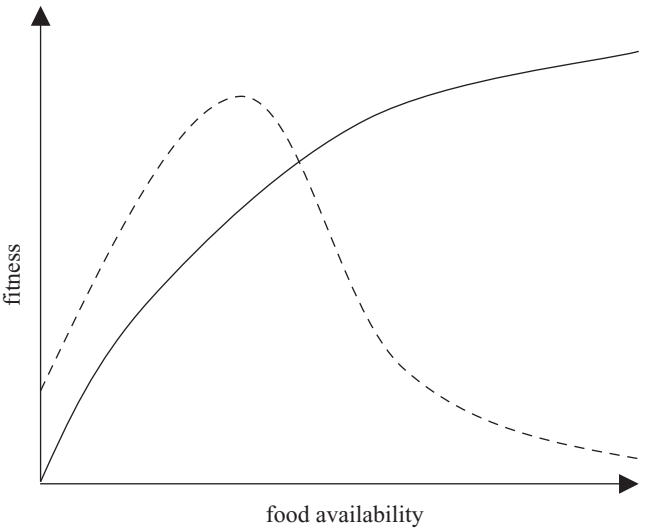

Figure 4. Schematic representation of variation in host (solid line) and parasite (dashed line) fitnesses as a function of food availability.

Consequently, preventing host emergence is in the interest of the parasite for its within-site transmission success. This provides an example of a positive correlation between a parasite's virulence and its transmissibility (Lipsitch & Moxon 1997; Dunn & Smith 2001).

(c) *Interrelationships of nutritional availability, host fitness and parasite fitness*

The significant increase in spore production as food increases indicates that the parasite took advantage of resources available to its host. The direct measure of spore production allowed us to disentangle the effects of environmental conditions on host and parasite. In low-food treatments, it is clear that the parasite varied its replication rate according to environmental conditions, as suggested by Thomas *et al.* (2002). This can be seen as an adaptation of the parasite to host conditions, since a higher reproductive rate and thus a higher resource-consumption rate in low-food conditions would risk killing the host before the parasite has produced its spores.

When we consider higher food levels, the influence of food level on spore production becomes weaker. This suggests that the growth rate of the parasite may be a positive saturating function of resource abundance.

Taken together, our results indicate that the correlation between host fitness and parasite fitness changes sign along the explored food gradient (figure 4). At low food levels host and parasite fitnesses are positively correlated: as nutritional availability increases, host fitness increases as both the probability of emergence and adult traits

positively correlated with fitness increase. Parasite fitness also increases because spore production increases more rapidly with resource availability than does the probability of emergence. The correlation between host fitness and parasite fitness becomes negative at higher food levels: as host fitness continues to increase with food availability, the parasite's fitness decreases because a large majority of infected mosquitoes emerge and thus the parasite's transmission, at least within larval sites, is strongly reduced.

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REFERENCES

Agnew, P., Bedhomme, S., Haussy, C. & Michalakis, Y. 1999 Age and size at maturity of the mosquito *Culex pipiens* infected by the microsporidian parasite *Vavraia culicis*. *Proc. R. Soc. Lond.* B **266**, 947–952. (DOI 10.1098/rspb.1999.0728.)

Agnew, P., Hide, M., Sidobre, C. & Michalakis, Y. 2002 A minimalist approach to the effects of density-dependent competition on insect life-history traits. *Ecol. Entomol.* **27**, 396–402.

Becnel, J. J. & Andreadis, T. G. 1999 The Microsporidia in insects. In *The Microsporidia and Microsporidiosis* (ed. M. Wittner), pp. 447–501. Washington, DC: ASM Press.

Bedhomme, S., Agnew, P., Sidobre, C. & Michalakis, Y. 2003 Sex-specific reaction norms to intraspecific larval competition in the mosquito *Aedes aegypti*. *J. Evol. Biol.* **16**, 721–730.

Briegel, H. 1990 Metabolic relationship between female body size, reserves and fecundity of *Aedes aegypti*. *J. Insect Physiol.* **36**, 165–172.

Brown, M. J. F., Loosli, R. & Schmid-Hempel, P. 2000 Condition-dependent expression of virulence in a trypanosome infecting bumblebees. *Oikos* **91**, 421–427.

Chambers, G. M. & Klowden, M. J. 1990 Correlation of nutritional reserves with a critical weight for pupation in larval *Aedes aegypti* mosquitoes. *J. Am. Mosquito Contr. Assoc.* **6**, 394–399.

Clements, A. 1992 *The biology of mosquitoes: development, nutrition and reproduction*. London: Chapman & Hall.

Colless, D. H. & Chellapah, W. T. 1960 Effects of body weight and size of blood-meal upon egg production in *Aedes aegypti* (Linnaeus) (Diptera, culicidae). *Ann. Trop. Med. Parasitol.* **54**, 475–482.

Dunn, A. M. & Smith, J. E. 2001 Microsporidian life cycles and diversity: the relationship between virulence and transmission. *Microbes Infect.* **3**, 381–388.

Ferguson, H. M. & Read, A. F. 2002 Genetic and environmental determinants of malaria parasite virulence in mosquitoes. *Proc. R. Soc. Lond.* B **269**, 1217–1224. (DOI 10.1098/rspb.2002.2023.)

Gilpin, M. E. & McClelland, G. A. H. 1979 Systems analysis of the yellow fever mosquito *Aedes aegypti*. *Fortschr. Zool.* **25**, 355–388.

Hausermann, W. & Nijhout, H. F. 1975 Permanent loss of male fecundity following sperm depletion in *Aedes aegypti*. *J. Med. Entomol.* **6**, 707–715.

Jokela, J., Lively, C. M., Taskinen, J. & Peters, A. D. 1999 Effect of starvation on parasite-induced mortality in a freshwater snail (*Potamopyrgus antipodarum*). *Oecologia* **119**, 320–325.

Kelly, J. F., Anthony, D. W. & Dillard, C. R. 1981 A laboratory evaluation of the microsporidian *Vavraia culicis* as an agent for mosquito control. *J. Invertebr. Pathol.* **37**, 117–122.

Lansdown, C. & Hacker, C. S. 1975 The effects of fluctuating temperature and humidity on the adult life table characteristics of five strains of *Aedes aegypti*. *J. Med. Entomol.* **11**, 723–733.

Lipsitch, M. & Moxon, E. R. 1997 Virulence and transmissibility of pathogens: what is the relationship? *Trends Microbiol.* **5**, 31–37.

Minchella, D. J. 1985 Host life-history variation in response to parasitism. *Parasitology* **90**, 205–216.

Motulsky, H. 1999 *Analysing data with GraphPad Prism*. San Diego, CA: GraphPad Software Inc.

Plaistow, S. J., Troussard, J.-P. & Cézilly, F. 2001 The effect of the acanthocephalan parasite *Pomphorhynchus laevis* on the lipid and glycogen content of its intermediate host *Gammarus pulex*. *Int. J. Parasitol.* **31**, 346–351.

Reynolds, D. G. 1970 Laboratory studies of the microsporidian *Pleistophora culicis* infecting *Culex pipiens*. *Bull. Entomol. Res.* **60**, 339–349.

Rice, W. R. & Chippindale, A. K. 2001 Intersexual ontogenetic conflict. *J. Evol. Biol.* **14**, 685–693.

SAS Institute 1997 *JMP v. 3.2.2*. Cary, NC: SAS Institute Inc.

Siva-Jothy, M. T. & Plaistow, S. J. 1999 A fitness cost of eugregarine parasitism in a damselfly. *Ecol. Entomol.* **24**, 465–470.

Sokal, R. R. & Rohlf, F. J. 2001 *Biometry*, 3rd edn. New York: Freeman.

Southwood, T. R. E., Murdie, G., Yasuno, M., Tonn, R. J. & Reader, P. M. 1972 Studies on the life budget of *Aedes aegypti* in Wat Samphaya, Thailand. *Bull. World Hlth Org.* **46**, 211–226.

Thomas, F., Brown, S. P., Sukhdeo, M. & Renaud, F. 2002 Understanding parasite strategies: a state-dependent approach? *Trends Parasitol.* **18**, 387–390.

Thompson, S. N. & Kavaliers, M. 1994 Physiological bases for parasite-induced alterations of host behaviour. *Parasitology* **109**(Suppl.), 119–138.

Trpis, M. & Hausermann, W. 1986 Dispersal and other population parameters of *Aedes aegypti* in an African village and their possible significance in epidemiology of vector-borne diseases. *Am. J. Trop. Med. Hyg.* **35**, 1263–1279.

Washburn, J. O. 1995 Regulatory factors affecting larval mosquito populations in container and pool habitats: implications for biological control. *J. Am. Mosquito Contr. Assoc.* **11**, 279–283.

Weiser, J. 1980 *Data sheet on the biological control agent* Vavraia (Pleistophora) culicis *(Weiser 1946)*. Geneva: World Health Organization.